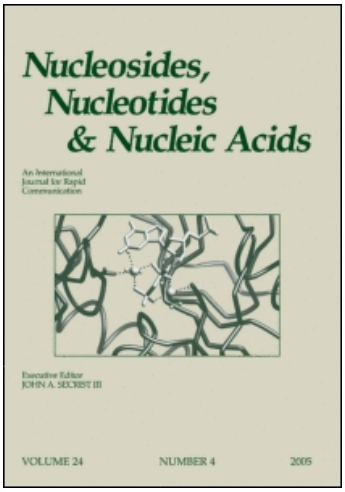

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SYNTHESIS AND CLONING OF A GENE ENCODING HUMAN INTERLEUKIN-1β (IL-1β)

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Abstract: The design, synthesis and cloning of a 466 base-pair DNA duplex coding for IL-1β is described.

Interleukin-1 (IL-1) represents a family of biologically active polypeptide hormones produced by activated macrophages. Members of the human IL-1 family play an important role in the pathogenesis of many diseases and function as key mediators of the host response to various infectious, inflammatory and immunological challenges. The cloning and expression of two human IL-1 cDNAs (IL-1α and IL-1β) have recently been described.[1-3] We now report the design, synthesis and cloning of a gene encoding human IL-1β and its expression in E.coli.

The synthetic construct was designed to provide the mature IL-1β protein based on the cDNA sequence described by Auron et al.[3] Its design incorporated the following additional features:

(1) an ATG translation initiation codon was placed at the start of the coding sequence;
(2) codons were optimised for expression in E.coli;
(3) the construct was scanned for unfavourable repeated sequences which were then altered within the constraints of the genetic code;
(4) the construct was also scanned for existing and potential restriction enzyme sites in order to assemble the gene in 4 portions;
(5) HindIII and EcoRI cohesive ends were added to the 5'- and 3'-termini respectively;
(6) KpnI and NcoI restriction sites were placed near the 5'-terminus to enable insertion into expression vectors and to facilitate alteration of the translation initiation region;
(7) a series of translation termination codons were placed at the end of

the coding sequence followed by a SalI restriction site for insertion into expression vectors.

The 4 portions of the gene were further divided into a series of overlapping oligomers which were synthesised by a number of automated solid-phase procedures:

(1) by phosphotriester methodology using 2-mesitylenesulphonyl chloride/1-methylimidazole as condensing agents on a Biosearch SAM1 DNA synthesiser;

(2) by methyl phosphoramidite methodology using tetrazole as condensing agent on an Applied Biosystems 380B DNA synthesiser;

(3) by β-cyanoethyl phosphoramidite methodology using tetrazole as condensing agent on a Biosearch 8600 DNA synthesiser.

Crude deprotected oligomers were purified by polyacrylamide gel electrophoresis (PAGE) and characterised by PAGE and autoradiography after 5'-phosphorylation with [γ-^{32}P]-ATP/T4 polynucleotide kinase. The appropriate labelled/phosphorylated oligomers of each portion were ligated using T4 DNA ligase and the double-stranded DNA of the correct size was gel-isolated and cloned into phage M13mp10.

The synthetic gene was removed from the phage vector M13mp10 on a NcoI-SalI fragment and inserted into a modified pBR322 vector, containing the Tac promoter, a synthetic ribosome binding site and a synthetic transcription terminator, to give plasmid pATTacIL1β/E. This plasmid was used to transform E.coli JM103 and RB791, both LacI^q strains. A similar procedure was used to give closely related plasmids bearing modified translation initiation regions. Following induction by isopropylthiogalactoside (IPTG), expression of Il-1β was demonstrated in the E.coli extracts by SDS-PAGE, Western blotting and bioassay.

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